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Inflammatory Markers as Predictors of Severity and Mortality in Coronavirus Disease: A Cross-sectional Study

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ABSTRACT

Introduction: COVID-19 is a pandemic that demands swift, in-depth, and well-grounded research work. WHO (World Health Organization) has declared around 1 million deaths and confirmed about 35 million cases worldwide.

Aim: To predict the role of three inflammatory markers (serum ferritin, C-reactive protein and lactate dehydrogenase) in severity and mortality in patients with corona virus disease patients admitted in the hospital.

Methodology: Patients with traceable SARS-CoV-2 by polymerase chain reaction (PCR) were inducted into this study by simple random sampling. Patients with potential radiological scans but without positive SARS were not included in this study. The nasopharyngeal sample technique was used to check the presence of the virus. This viral load's severity was checked via World Health Organization recommendations. All the information including the sociodemographic data, severity of disease and laboratory values of three inflammatory markers gathered on a semi structured proforma designed for the study. Study Design: A Cross-Sectional study. Place and Duration: Isra University Hospital from 1st June 2021 to 31st December 2021.

Results: A 240 patients were included in the study; aged between 20 and 60 years. The values of the three inflammatory markers were found raised in the group of patients being studied (more frequently within the moderate and severe disease groups). C - reactive protein was raised in 122 patients, ferritin was raised in 83 patients and Lactate dehydrogenase was raised in 184 patients

Conclusion: Measuring the values of CRP, LDH, and Ferritin at admission can be a reliable predictor of severity and mortality in SARs-CoV-2. Out of the three, ferritin is the best indicator and should be considered essential.

Key Words: Coronavirus disease, Mortality, Ferritin, CRP, Lactate dehydrogenase, Inflammatory

INTRODUCTION

COVID-19 is a pandemic that demands swift, in-depth, and well-grounded research work. The in-hospital presentation shows off an entire spectrum of symptoms in the affected population; from asymptomatic patients to patients with complete respiratory failure. The World Health Organization has declared around 1 million deaths and confirmed about 35 million cases worldwide.[1] According to the Pakistani Government, in Pakistan COVID-19 mortality rate has been recorded at 2.06%.[2] Inflammatory markers are important in the assessment of this viral disease as inflammation plays an important role in its pathogenesis.

When white blood cells in the body get activated as a result of viral infiltration, they release a large number of inflammatory chemokines and cytokines which in turn leads to the permeation of monocytes and neutrophils inside the lungs. As a result of which, the endothelium and the alveolar cells are damaged. This state of bodily conditions is termed acute respiratory distress syndrome or tissue hypoxia.[3] C-reactive protein gets elevated in a COVID-19 infection and triggers a protective response in the body. This response allows it to

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bind itself with phosphocholine present on the surface of affected cells, which in turn activates the classical complement pathway and ejects the virus as well as the damaged cells from the host.[4]

Cytokine storms are the foremost reason for mortality in cases of severe disease. Interleukin-6 (one of the two inflammatory contributors) is the main protagonist. Unfortunately, in most Pakistani setups it cannot be routinely measured.[5] C-reactive protein can instead be used as a substitute for reliable interleukin-6 testing, to measure these cytokine release storms.[6] Inflammation is the main cause of death among patients as it can cause worsening of the respiratory function to the point where complete shut-down can occur in less than four days.[7] In order to avoid this unfortunate situation, proper post-hospital care and monitoring can be instituted which can let the physicians keep track of the disease and introduce efficient and effective interventions in severe to critical patients.

Many studies have been done in order to check the effectiveness of various inflammatory markers for COVID-19. Most of these markers that rank high in terms of reliability and effectiveness are not easily available and are also economically unfeasible. The three markers that were chosen for this study, serum ferritin, C-reactive protein, and lactate dehydrogenase are accessible at almost all laboratory settings. If certainty can be achieved in terms of the results of these three markers then this could mean that customized care is possible and can be given to individual patients to a great standard.

COVID-19 is a disease that is relatively in its early period when it comes to the level of research done and meaningful/applicable medical literature gathered. Despite having accumulated a praise-worthy level of theoretical knowledge in terms of the disease itself, professional practitioners are unfortunately still lacking solid local data which could give concreteness to the inter-relationship of inflammatory markers and mortality. This piece of work is dedicated to establishing the reliability of the three inflammatory markers (namely serum ferritin. C-reactive protein and lactate dehydrogenase at hospital admission of infected COVID-19 patients for mortality, and to establishing a definitive basis for best cutoff values for each marker.

METHODOLOGY

This is a cross-sectional study and was conducted at Isra University Hospital from 1st June 2021 to 31st December 2021. The protocol for this study work was approved and allowed by the Ethics Review Committee of the said hospital. During the time period mentioned above the patients that had a confirmed case of the infection were considered valuable for study by simple random sampling. Consent was not taken from the patients as it was not considered a prerequisite for a study that was to publish the data anonymously in terms of the individual patient/participant. The diagnosis was made based on a positive polymerase chain reaction (PCR). Patients with potentially indicative radiological scans but without positive SARS test results (on more than one occasion) were specifically not included in the study.

During the initial hit, the hospital data showed the mortality rate to be below 10%. A very recent study conducted in Pakistan showed the maximum AUC (Area under Curve) to be 0.875 for all three inflammatory markers.[8] Keeping all the above in mind the following study group was considered.

As per the institution's protocol, a nasopharyngeal swab method for polymerase chain reaction (PCR) was used to ascertain the presence of the contagious viral infection in all patients prior to their admission to the hospital. The results were recorded qualitatively; either present or absent. Blood samples for the quantitative measurement of C-reactive protein, Ferritin, and LDH were taken within the first 30 minutes' post-admission and were sent to Isra University Hospital laboratory. After the patients were discharged or post mortality the records for a total duration of in-hospital stay were also collected from the medical archives for the purpose of this study paper. Hospital's lab information management software network was used in case of missing reports. After collecting and compiling all the hospital data against each individual patient, the disease severity for each case was marked using the World Health Organization assessment charts.[9] As the number of patients falling within the critical disease category was meagre, this category was merged with the severe disease category.

Table I: Severity analysis of SARS-Cov-2 viral infection (adjusted to suit version of World Health Organization's clinical management guidelines).

Category	Definition
Mild	Basic definitive symptoms of COVID-19 are showcased
Moderate	Evident symptoms of pneumonia (fever, dyspnea, cough) + no symptom of critical pneumonia
Severe	Evident symptoms of pneumonia (fever, dyspnea, cough) + either; respiratory rate >30 breaths/min; or SpO2 <90% on room air; severe respiratory distress
Critical	Acute respiratory distress syndrome

System IBM SPSS Statistics 20.0 Version was used for data analysis (IBM Corp, Armonk, NY). Data were expressed in the following two ways, Qualitative data: in terms of percentages and numbers/numeral figures and Quantitative data: in terms of mean±standard deviation.

Interquartile range and median were used for variables that were continuous (interpolated) with the non-parametric

distribution. Shapiro-Wilk test was used to check the normality of the data collected. All three categories of patients in terms of severity (keeping in mind that the fourth category 'critical disease' was made a part of the third 'severe disease' category) were tested using the Kruskal-Wallis method for the purpose of drawing comparisons between the interquartile and median ranges for the three inflammatory markers.

Using the two outcome-based groups as a basis, levels of C-reactive protein, serum LDH, and serum ferritin were compared via independent-samples by Mann-Whitney U-test. Another indispensable test namely the Chi-square/ Fisher's exact test was used to compare the proportionality of patients (of separate groups) with high ranges of inflammatory markers against each other. After all these tests were applied, the ROC (receiver operating characteristic) curve analysis was done to measure both the specificity and the sensitivity of CRP, LDH, and Ferritin as predictors of mortality. This curve was also used to determine their cut-off values in order to ascertain and justify the most appropriate times for intervention. IBM SPSS Statistics (the system previously referred to) automatically generated the AUC (area under the curve) values for each marker, hence helping with the study. This parameter throws light on how significantly efficient the statistical model is at differentiating. For instance, if the differentiation between the number of people dying in-hospital or getting discharged is good, then the model would show a raised value for AUC. In order to calculate the probability of the patients getting discharged, the study used the Kaplan Meier methodology. $p < 0.05$ was the value taken as significant for all the comparisons.

RESULTS

During the time interval considered for this study paper, about 450 infected patients were admitted. Out of the above, 9% died (about 39 admitted patients). 98 patients out of the total 450, had incomplete data sets (due to various reasons). 70 patients had probable COVID-19 and 3 patients were left without proper medical clearance. All of these mentioned above were not included in the data evaluation phase. Clinical fit-for-evaluation characteristics of the rest of the patient group are included in the study displayed in Table II. The mean age was 40±15 and 87.13% were males. Mild cases of COVID-19 disease successfully fought against the odds of mortality and are 160 (66.39%) in number with total death reported in 20 (8.29%) patients due to severe disease. Table III show the exact values of three inflammatory markers that were calculated (after blood testing) for the population under study. Serum ferritin is highly sensitive with sensitivity of 95.45% and C reactive protein is highly specific with specificity of 88.89% among all three inflammatory markers (see Table IV).

The values for these inflammatory markers were found to be above the higher limits (limit considered normal) in the greater proportion of the affected that had disease ranging from moderate to severe to critical to those who died. The results of the Kaplan Meier analysis for affected patient survival at the above-mentioned thresholds are displayed in Table IV.

Patient's CRP, ferritin and LDH improved in those who were discharged, while it increased significantly in those who were died (As shown in Table 5)

Table II: Clinical characteristics of population under study.

Variable		Value
Age		40 ± 15
Gender	Male	210 (87.13%)
	Female	31 (12.86%)
Severity of disease at the time of admission	Mild	160 (66.39%)
	Moderate	40 (16.59%)
	Severe	41 (17.01%)
Mortality	Total	20 (8.29%)
	Due to mild illness	0 (0%)
	Due to moderate illness	0 (0%)
	Due to severe illness	20 (100%)

Table III: Values of the three inflammatory markers for the population under study.

Inflammatory Marker	Elevated Values in % of Patients
Serum CRP	51.26 %
Serum Ferritin	34.87 %
Serum LDH	77.31%

Table IV: Sensitivity and specificity of inflammatory markers

Inflammatory Marker	AUC + 95% Confidence Interval (Figure 3)	Sensitivity	Specificity
C-reactive protein ≥45.5 mg/L	0.909 (0.854-0.964)	86.36%	88.89%
Ferritin ≥723 ng/ml	0.915 (0.835-0.995)	95.45%	86.57%
LDH ≥428.5 U/L	0.863 (0.785-0.942)	90.91%	80.56%

Table V: Different groups of patients against different inflammatory markers (CRP, ferritin, LDH).

	Total Patients (n=240)	Outcomes			Severity of Disease (In-Hospital)			
		Discharged (n=220	Death (n=18)	P	Mild (n=160)	Moderate (n=34)	Severe (n=44)	P
CRP (mg/l)	6.1 (2.1-29.1)	4.5 (2.0-21.0)	120.5 (74.7-180.0)	<0.001	3.0 (1.5-7.5)	24.5 (5.1-53.2)	91.0 (27.3-146.5)	<0.001
Ferritin (ng/ml)	232.0 (118.0-603.7)	215.0 (109.0-439.2)	1759.5 (1198.7-2100.5)	<0.001	171.0 (98.0-278.5)	471.5 (261.7-837.5)	1275.9 (611.5-1915.0)	<0.001
LDH (U/l)	300.0 (227.6-435.6)	288.0 (225.0-402.2)	640.0 (533.5-804.5)	<0.001	265.0 (221.0-352.0)	409.0 (263.7-600.5)	493.0 (349.5-712.5)	<0.001

Only interquartile and median range values are shown above.

DISCUSSION

All the results compiled above in various formats clearly show that patients that have contracted COVID-19 in moderate to severe levels have a higher level of inflammation in their bodies versus patients that have contracted the disease in a milder form. These results are in accordance with the majority of the other current studies' conclusions. It is a well-established fact by now that cytokine storms are a telltale sign (pathophysiological model) of the severe form of the considered disease [10]. One thing to notice though is that even this study marks inflammatory markers as high in serious cases of COVID-19, it does not differentiate between the levels of markers when it comes to moderate and severe cases. This last statement sits in contrast to what is available in the medical literature regarding COVID-19; which is that the higher the criticalness of a disease, the higher the inflammation, hence the higher the levels of inflammatory markers.[11] A reason for this could be that if we place data against a continuous spectrum, it could place higher levels of disease against higher levels of inflammatory markers as both would move progressively towards the upper sections of the spectrum. Nonetheless, according to the current practice of physicians worldwide, patients towards this higher spectrum (moderate to severe level) are advised to stay in the hospital while milder disease-inflicted patients are recommended to continue treatment at home.[12] Another thing to note here is that this study showed no variance between the levels of C-reactive protein in severe cases and in moderate ones [13].

If the inflammatory markers are considered individually, we can see that serum ferritin is the only contender that shows the least level of quantitative interference when it comes to patients falling within the moderate to severe disease range. Yet, the study results also show that serum ferritin is also the only contender when it comes to predicting mortality from the beginning as it has the greatest odds ratio and well the highest AUC (area under the curve). Hence, when the patients showed a high level of ferritin in their bloodstream, it could be concluded that mortality will be high.

Why is the level of ferritin so high in COVID-19? The answer is believed to be either the production of high levels of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6) in the body or the increased release from damaged cells in the bloodstream.[14] The case for serum ferritin as a dependable predictor for mortality is fought differently by different studies. (Feld et al.). Serum ferritin is a poor predictor at admission (AUC - 0.677).[15] (Harold et al.) Serum ferritin is a relatively reliable predictor (compared to above) with AUC at 0.750 [16]. The cut-off will be set at 1285 ng/ml; time for the introduction of mechanical ventilation.[17] Elevated levels of ferritin were directly proportional to the likelihood of death in New York. (This result is consistent with the current study conclusions.)

The study at hand concludes that if the value of C-reactive protein (CRP) increases seven-folds, then the case for mortality prediction is solid. In order to avoid various complications that can occur before and can lead to death, a number of cut-off values are set for CRP. The results of different studies include the following conclusive discussions: Huang et al. If CRP ≥10mg/L, the risk of death increases threefold in patients.[18] Liu et al. If CRP > 41.8 mg/L, acute complications are expected.[19] Luo et al. CRP >41.4mg/L is 95% sensitive as a predictor as well as 77.6% specific when it comes to death in hospitalized patients.[20]

COVID-19 is not the only viral infection to have raised levels of LDH. Other viral diseases such as H5N1 and MERS-CoV also result in increasing LDH levels.[21] If the level of LDH rises by two, then it becomes a very surefire marker of death in the hospital cohort. If LDH levels ≥1200 U/L then according to an American study, one is eight times more susceptible to death than one with levels less than 1200 U/L.

A local study (in Pakistan) done by Asghar et al. took under the microscope 360 indoor patients afflicted by COVID-19.[8] This study was done for the purpose of measuring a large number of parameters. Fortunately, this set of parameters also included the three inflammatory markers. All three markers showed lower values when compared to the results of the study at hand. Surprisingly, they deduced serum ferritin as the

weakest mortality predictor. Despite this interesting inference, we would still encourage the use of ferritin as the first priority.

The distribution of males and females per each class of severity was unequal in this study despite as per fact that males overall greatly outnumbered the females [22]. This is not surprising as the data from Global Health 50/50 Research Initiative also shows a percentage as high as 74% of affected population in Pakistan to be only males.[23] This is not the case with European countries. Their datasets show the number of male and female confirmed case affected to be approximately equal in number.[24] This discrepancy could be because of the conservativeness of our society which renders the females to be less outgoing and hence contracting less disease.

One thing to note, which makes this dataset consequential, is that the blood sampling for all patients was done strictly within the first 30 minutes post-admission. This was because of the hospital protocol. Consequently, in order to avoid partiality, patients who could not be assessed within this time period upon admission were entirely excluded from the study population. For this study, the authors studied the inflammatory markers only in the beginning. It would be fascinating to know how these markers fluctuate throughout the stay of the patient; how hospitalization affects the results. Even though some inflammatory markers are already established as reliable, such as interleukin-6 and procalcitonin, the authors could not test them due to a number of reasons, most of which were of a financial nature. This was a period of intense workload for the participants performing the analysis. For this reason, no standardized textbook format was used to document the data. The negative impact of this was that the authors could not include within this research the study of the factors that adversely affect the levels of the three inflammatory markers, specifically CRP. One such example is the patients with cirrhosis. Their CRP levels are naturally lower than the rest. Another example consists of those patients that are on medications like statins.[25] They also have a decreased level of CRP in their blood.

CONCLUSION

Serum C-reactive protein, LDH, and Ferritin if measured at the time of admission can prove to be reliable predictors of severity and mortality in COVID-19 disease. Out of the three, ferritin is highly sensitive and should be considered essential.

Funding Source

None.

Conflict of Interest

None.

Permission

Permission was taken from the ethical review committee of the institute.

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